1122-170 Expression of Tob by Human Coronary Arteries and BMP-Mediated Stimulation of Coronary Artery Endothelial Cell Proliferation

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Background: Little is known regarding the role of bone morphogenetic protein (BMP) signaling that control vascular cell growth during normal homeostasis and in arterial pathologies, notably atherosclerosis and arterial calcification. Here we tested the hypotheses that Tob, an antiproliferative protein that negatively regulates BMP signaling, is expressed in human coronary arteries and in cultured human coronary artery endothelial cells (ECs) and that BMP2 stimulates proliferation in cultured ECs. **Methods and Results:** Immunohistochemical localization showed abundant expression of Tob in ECs of normal human arteries. We therefore determined expression of Tob and BMP receptor (BMPR)-IA, -IB, and II in cultured ECs at baseline and stimulated with BMP2 (300 ng/mL for 6 hr). Experiments were also performed in the presence of the proteasome inhibitor epoxomicin that enhances BMP signaling by preventing degradation of BMP signaling pathway components. ECs expressed Tob and BMPR-IA and -II, but did not express BMPR-IB. Stimulation with either BMP2 or epoxomicin resulted in a significant increase in expression of BMPR-IA and BMPR-II ($p < 0.01$ for both BMP2 and epoxomicin), but there was no change in Tob mRNA. Stimulation of ECs with either BMP2 or epoxomicin significantly increased proliferation measured by both a colorimetric proliferation assay ($p < 0.001$ for both BMP2 and epoxomicin; $n = 5$) and by proliferating cell nuclear antigen (PCNA) assay ($p < 0.0.05$ and 0.006; $n = 3$) after 48 hr in a dose-dependent manner. Toxicity was observed at high doses of epoxomicin. **Conclusions:** The antiproliferative BMP pathway inhibitor Tob and BMPR-IA and II are abundantly expressed by human coronary artery ECs both in vitro and in vivo. Expression of BMPR but not Tob transcripts are affected by both BMP2 and epoxomicin. BMP2 may regulate EC proliferation, suggesting a possible role in the growth of ECs during normal homeostasis and in arterial pathologies involving EC denudation or proliferation. Since Tob has been implicated as an antiproliferative regulator in many other cell types, it may also regulates EC proliferation via a mechanism that is not dependent on altered transcription of Tob in response to BMP stimulation.

1122-171 Atherogenic Low-Density Lipoprotein Impairs Vascular Endothelial Cell Survival by Disrupting the FGF2-PI3K-Akt Autoregulatory Loop

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Background:
Atherogenic LDL, such as circulating electronegative LDL and oxidized LDL (oxLDL), can inhibit proliferation and induce apoptosis in vascular endothelial cells (EC). Fibroblast growth factor 2 (FGF2) stimulates phosphatidylinositol 3-kinase (PI3K), which in turn activates Akt, a protein kinase that regulates cell survival. This study was designed to investigate how oxLDL interferes with signal transduction along the FGF2-PI3K-Akt pathway.
Methods:
The interrelationship between FGF2 and Akt was examined in cultured bovine aortic EC (BAEC). To investigate further the role of endogenous FGF2 in EC survival, BAEC[FGF2(+)] and BAEC[FGF2(–)] cell lines were established by stable transfection of BAEC with FGF2 sense and antisense cDNAs.
Results:
In cultured BAEC, oxLDL (50 μg/mL) inhibited FGF2 transcription and Akt phosphorylation, leading to marked apoptosis. Consistent with the cell-survival properties of Akt, PI3K inhibitor wortmannin (25-200 nM) also inhibited FGF2 expression and induced apoptosis in a concentration-dependent manner. Stable overexpression of FGF2 in BAEC[FGF2(+)] greatly enhanced Akt phosphorylation, rendering the cells resistant to oxLDL. In contrast, deprivation of endogenous FGF2 in BAEC[FGF2(–)] led to reduced Akt phosphorylation and enhanced spontaneous apoptosis. Inhibition of FGF2 protein synthesis by the antisense RNA also led to inhibition of FGF2 transcription in BAEC[FGF2(–)], suggesting that endogenously produced FGF2 may be the most important stimulator of its own induction in the low-serum, mitogen-free culture system. Furthermore, G2/M transition in the cell cycle and DNA synthesis were severely inhibited in BAEC[FGF2(–)], limiting proliferation. In contrast, unlike the parental BAEC, BAEC[FGF2(+)] were resistant to the inhibitory effects of oxLDL on G2/M transition and DNA synthesis.
Conclusion:
EC survival depends on continuous activation of the PI3K-Akt pathway by endogenous FGF2, which is required for its own induction in the manner of an autocrine. Maintenance of the integrity of the FGF2-PI3K-Akt autoregulatory loop is essential for EC survival in the presence of atherogenic lipoproteins including oxLDL.

1122-172 Homocysteine Induced Endothelial Cell Damage Through NF-κB Activation and Monocyte Chemoattractant Protein-1 and Vascular Cell Adhesion Molecule-1 Expression

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Background & Aim: Homocysteine is known to damage endothelial cells by oxidative stress, most studies were however performed with excessively high concentration. In order to evaluate the action mechanism of homocysteine in clinically relevant concentration, S-adenosylhomocysteine (SAH) was induced intracellularly and NF-κB, the important transcriptional regulator for oxidative stress, and its downstream mediators, MCP-1 and VCAM-1 expressions, were evaluated.
Methods: SAH was formed in human umbilical vein endothelial cells (HUVECs) by administering homocystine, adenosine, and erythro-9-(2-hydroxy-3-nonyl) adenine to the culture media and HUVECs were incubated for 72 hours. Intracellular reactive oxygen species (ROS) formation was quantified by fluorescent intensity of dichlorofluorescin (DCF) with confocal microscope. The proliferation and survival of HUVECs were evaluated by [^{3}H]-Thymidine uptake and MTT assay. NF-κB activity, MCP-1 secretion and VCAM-1 expression were assayed by EMSA, ELISA and Western blot respectively.
Results: HUVECs showed atrophic change after intracellular SAH formation and the deterioration was progressive according to time and concentration.
Intracellular ROS production evaluated by DCF fluorescence was increased. The cell proliferation rates evaluated by [^{3}H]-Thymidine uptake were decreased both in 40μM SAH group (148 ± 47 CPM) and 200μM SAH group (139 ± 50 CPM) (control=207 ± 22 CPM) and the cell survival rates assayed by MTT uptake were also decreased both in lower and higher SAH groups (156 ± 12 CPM and 106 ± 13 CPM) compared with control (185 ± 10CPM).
NF-κB was activated by SAH induction, which was followed by increased MCP-1 secretion and VCAM-1 expression.
Conclusion: We have shown that homocysteine damaged endothelial cells even in clinically relevant concentrations through sustained exposure with intracellular SAH formation. And intracellular ROS production and NF-κB activation were observed and MCP-1 secretion and VCAM-1 expression might lead to inflammatory response in SAH-treated endothelial cells. These data identified novel mechanism of homocysteine inducing endothelial cell damage.

1122-173 Changes in Innate and Adaptive Humoral Immune Responses and Indices of Atherosclerosis in Aging

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Background: Immunization against atherosclerosis is a promising therapy but the natural course of immune responses against oxLDL during aging is not known. We hypothesized that aging alters innate or adaptive immune responses to oxLDL modulating the progression of atherosclerosis and plaque phenotype in apoE -/- mice. **Method:** Mice on Western diet were sacrificed at 15-17, 36 or >50 weeks of age. Descending aorta was stained en-face for lipids. Plaque lipid, macrophage and collagen content were evaluated in the aortic sinus. Innate immune response was assessed using anti Cu-oxLDL and anti phosphorylcholine (PC) IgM and adaptive immune response to oxLDL was assessed using anti MDA-LDL and Cu-oxLDL IgG titers. Splenic cytokines were evaluated using RT-PCR. **Result:** (Table) Aging was associated with increased atherosclerotic burden and collagen content with decreased macrophage and plaque lipid. MDA-LDL IgG increased in the 36 weeks group but reduced in mice >52 weeks. Cu-oxLDL and PC-IgM increased significantly with age with cross-reactivity to each other. Cu-oxLDL IgG increased with age with no isotype specificity. Splenic T-helper cytokine mRNA expression also increased with age. **Conclusion:** Innate immune response as indicated by antibody titers to CuoxLDL and PC is associated with increased plaque sizes and a more stable phenotype.

Age	15-17 weeks	36 weeks	>52 weeks
% En-face area	0.5±0.3 n=6	24.1±4.3‡ n=8	46±13.5‡¶ n=6
Sinus plaque (mm sq)	0.31±0.06 n=5	0.65±0.1‡ n=5	1.13±0.14‡¶ n=6
% Plaque Lipid	29.6±7.5 n=6	20.5±4.7‡ n=8	11.1±4.2‡¶ n=6
% Macrophage	14.2±4.5 n=6	10.2±2.7 n=8	5.1±3.1‡¶ n=6
% Collagen	27.7±7.1 n=5	38.4±3.0 n=7	43.4±14‡ n=6
MDA-LDL IgG	0.063±0.024	0.129±0.036‡	0.064±0.044¶
Cu-oxLDL IgG	0.125±0.036	0.435±0.201	1.049±0.560‡¶
Cu-oxLDL IgM	0.316±0.070	0.560±0.286	1.011±0.356‡
PC IgM	0.419±0.068	0.628±0.276	0.923±0.308‡
IL-4† n=4	0.008±0.004	0.023±0.005	0.062±0.029‡¶
IFN-γ€ n=4	0.015±0.006	0.04±0.014	0.128±0.095‡
IL-10† n=3	0.025±0.018	0.113±0.175	0.247±0.109

†Densitometric units relative to β-actin; ¶p<0.05 vs. 36 wk; ‡p<0.05 vs. 17 wk; IgG and IgM ELISAs n=5 for 15-17 and 36 weeks and n=6 for >52 weeks; expressed as OD 405

1122-174 Thrombin and Histamine Stimulate Phosphorylation of Endothelial Nitric Oxide-Synthase via an Akt-Independent, AMP-Activated Kinase-Dependent Pathway

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Background: The protein kinase Akt is involved in vascular development and several endothelial functions, including activation of endothelial NO-synthase (eNOS) and promotion of endothelial cell survival. Recently we have found that although Akt-phosphorylation is inhibited by the G-protein activators thrombin and histamine these agonists stimulate phosphorylation of eNOS on Ser1179. The purpose of this study was to examine the role of other protein kinases in mediating this Akt-independent phosphorylation of eNOS.